

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification 5 : B01J 19/00, C07H 21/00, C03C 17/30, C07B 61/00, C07K 1/04</p>	<p>A1</p>	<p>(11) International Publication Number: WO 94/27719 (43) International Publication Date: 8 December 1994 (08.12.94)</p>
<p>(21) International Application Number: PCT/US94/05896 (22) International Filing Date: 25 May 1994 (25.05.94) (30) Priority Data: 08/068,540 27 May 1993 (27.05.93) US (71)(72) Applicant and Inventor: BRENNAN, Thomas, M. [US/US]; Apartment 705, 2000 Broadway, San Francisco, CA 94115 (US). (74) Agent: McDONNELL, John, J.; Allegetti & Witcoff, Ltd., Ten South Wacker Drive, Chicago, IL 60606 (US).</p>	<p>(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report. AML INFORMATION SERVICES P O BOX 405, CORTE MADERA, CA 94976-0405 (415) 927-0340 • FAX (415) 927-7250</p>	
<p>(54) Title: METHOD AND APPARATUS FOR CONDUCTING AN ARRAY OF CHEMICAL REACTIONS ON A SUPPORT SURFACE</p> <p>(57) Abstract</p> <p>The invention provides apparatus and methods for making arrays of functionalized binding sites on a support surface. The invention further provides apparatus and methods for sequencing oligonucleotides and for identifying the amino acid sequence of peptides that bind to biologically active macromolecules, by specifically binding biologically active macromolecules to arrays of peptides or peptide mimetics.</p>		

METHOD AND APPARATUS FOR CONDUCTING AN ARRAY OF
CHEMICAL REACTIONS ON A SUPPORT SURFACE

BACKGROUND OF THE INVENTION

5 This is a continuation-in-part of Serial No. 07/754,614 filed September 4, 1991, pending.

Field of the Invention

The invention relates to methods for conducting a large number of chemical reactions on a support surface, methods for making the support surface, and the support surface itself.

Summary of the Related Art

Proposals for the direct sequencing of DNA by hybridization with arrays of oligonucleotides are known in the art. Drmanac et al., Genomics 4: 114 (1989) proposes hybridization array-mediated DNA sequencing by binding target DNA to a dot blot membrane, followed by probing with an array of oligonucleotides. Khrapko et al., FEBS Letters 256, 118 (1989) proposes hybridization array-mediated DNA sequencing by binding the oligonucleotide array to a support membrane, followed by probing with target DNA.

Synthesis of arrays of bound oligonucleotides or peptides is also known in the art. Houghton, in the Multiple Peptide System product brochure describes the T-bag method, in which an array of beads is physically sorted after each interaction. This method becomes unwieldy for the preparation of large arrays of oligonucleotides. Geysen et al., J. Immunol. Methods 102: 259 (1987) discloses the pin method for the preparation of peptide arrays. The density of arrays that may be produced by this method is limited, and the dipping procedure employed in the method is cumbersome in practice. Southern, Genome Mapping and Sequencing Conference, May 1991, Cold Spring Harbor, N.Y., disclosed a scheme for oligonucleotide array synthesis in which selected areas on a glass plate are physically masked and the desired chemical reaction is carried out on the unmasked portion of the plate. In this method it is necessary to remove old mask and apply a new one after each interaction. Fodor et al., Science 251: 767 (1991) describes a method for synthesizing very dense 50 micron arrays of peptides (and potentially oligonucleotides) using mask-directed photochemical deprotection of synthetic intermediates. This method is limited by the slow rate of photochemical deprotection and by the susceptibility to side reactions (e.g., thymidine dimer formation) in oligonucleotide synthesis. Khrapko et al, FEBS Letters 256: 118 (1989) suggests simplified synthesis and immobilization of multiple

oligonucleotides by direct synthesis on a two dimensional support, using a printer-like device capable of sampling each of the four nucleotides into given dots on the matrix. However, no particulars about how to make or use such a device are provided.

5 Some methods for permanently attaching oligonucleotides to glass plates in a manner suitable for oligonucleotide synthesis are known in the art. Souther, Chem. abst. 113: 152979r (1990) describes a stable phosphate ester linkage for permanent attachment of oligonucleotides to a glass surface. Mandenius et al., Anal. Biochem. 157: 283 (1986) teaches that the hydroxyalkyl group resembles the 5'-hydroxyl of
10 oligonucleotides and provides a stable anchor on which to initiate solid phase synthesis.

 The related art contains numerous ideas and information related to arrays of chemical reactants on a solid support. However, existing or suggested methods are limited, and do not conveniently and reliably produce the very large, high density
15 arrays. There is, therefore, a need for new methods for preparing large high density arrays of reactive sites. Ideally, such methods should utilized relatively simple machinery to produce large, dense arrays of solid phase bound reactants in a reproducible and rapid manner.

SUMMARY OF THE INVENTION

This invention provides a method for conducting a large number of chemical reactions on a support surface. Solutions of chemical reactants are added to functionalized binding sites on the support surface by means of a piezoelectric pump. This pump deposits microdroplets of chemical reactant solution onto the binding sites. The chemical reactant at each binding site is separated from the others by surface tension. Typically, the support surface has 10^3 - 10^4 functionalized binding sites per cm^2 and each functionalized binding site is about 50-2000 microns in diameter. Typically, the amounts of reagents added to each binding site is in a volume of about 50 picoliter to 2 microliter. The reactions at the functionalized binding site may form covalent bonds such as esters or amide bonds or may involve non-covalent specific binding reactions such as antibody/antigen binding or oligonucleotide specific binding. The invention also includes array plates and methods for making the array plates.

Typically, the array plates are made by the process set out in Figure 2A by

- (a) coating a support surface with a positive or negative photoresist substance which is subsequently exposed and developed to create a patterned region of a first exposed support surface;
- (b) reacting the first support surface with a fluoroalkylsilane to form a stable fluoroalkylsiloxane hydrophobic matrix on the first support surface;
- (c) removing the remaining photoresist to expose a second support surface; and
- (d) reacting the second support with a hydroxy or aminoalkylsilane to form derivatized hydrophilic binding site regions.

The preferred siloxane reaction product of the present invention is tetradecafluoro-1,1,2,2-tetrahydrooctyl siloxane. In Figure 2A, the hatched lines are the solid support, "S1" represents a first exposed support surface site, "S1-F" is a hydrophobic fluoroalkylsilane site, and "S1-OH" is a derivatized hydrophilic binding site.

Alternatively, the array plates can be made by the process set out in Figure 2B by

- (a) reacting a support surface with a hydroxy or aminoalkylsilane to form a derivatized hydrophilic support surface;
- (b) reacting the support surface from step (a) with o-nitrobenzyl carbonyl chloride as a temporary photolabile blocking to provide a photoblocked support surface;

- (c) exposing the photoblocked support surface of step (b) to light through a mask to create unblocked areas on the support surface with unblocked hydroxy or aminoalkylsilane;
- (d) reacting the exposed surface of step (c) with perfluoroalkanoyl halide or perfluoroalkylsulfonyl halide to form a stable hydrophobic (perfluoroacyl or perfluoroalkylsulfonamido) alkyl siloxane matrix; and
- (e) exposing this remaining photoblocked support surface to create patterned regions of the unblocked hydroxy- or aminoalkylsilane to form the derivatized hydrophilic binding site regions.

10 The preferred siloxanes of the present invention are 3-perfluorooctanoyloxy propylsiloxane and 3-perfluorooctanesulfonamido propylsiloxane. In Figure 2B, the hatched lines are the solid support, "-A" represents a hydrophilic support site, "-A B" represents a temporary photolabile blocked support site, and "-A F" represents a hydrophobic site.

15 The invention also provides a method for determining or confirming the nucleotide sequence of a target nucleic acid. The target nucleic acid is labelled by conventional methods and hybridized to an oligonucleotides of known sequence previously bound to sites on the array plate. The array plate having bound labelled target nucleic acid is then washed at appropriate stringency and the presence and

20 location of bound labelled target nucleic acid is determined using scanning analyzers. Since the sequence of the covalently attached oligonucleotide in each element on the array is known, this allows the unambiguous determination of the nucleotide sequence of the target nucleic acid.

The methods of the invention may also be applied to the determination of

25 peptides or peptide mimetics that bind biologically active receptors. In this aspect, peptide arrays of known sequence can be applied to glass plates using the same piezoelectric pump/surface tension wall method described *supra*. The resulting array of peptides can then be used in binding analyses with biologically active receptor ligands to screen for peptide mimetics of receptor agonists and antagonists. Thus,

30 the invention provides a method for producing peptide array plates, peptide array plates having covalently bound peptides separated by surface tension areas, and methods of using such peptide array plates to screen for peptide mimetics of receptor agonists and antagonists.

Those skilled in this art will recognize a wide variety of binding site and

35 chemical reactants for forming either covalent bonds or for specific binding reagents.

BRIEF DESCRIPTION OF THE DRAWINGS

- Figure 1: Hybridization analysis using arrays of trimers. Individual dots that have bound the DNA fragment are underlined.
- Figure 2A: Illustrates the formation of an array surface that is ready for solid phase synthesis.
- Figure 2B: Illustrates O-Nitrocarbamate array making chemistry.
- Figure 3: Surface tension wall effect at the dot-interstice interface. The droplet containing solid phase synthesis reagents does not spread beyond the perimeter of the dot due to the surface tension wall.
- Figure 4: Hydrogen-phosphonate solid phase oligonucleotide synthesis on an array surface prepared according to Example 1.
- Figure 5: Top and side views of a piezoelectric impulse jet of the type used to deliver solid phase synthesis reagents to individual dots in the array plate synthesis methods according to the invention.
- Figure 6: Use of a piezoelectric impulse jet head to deliver blocked nucleotides and activating agents to individual dots on an array plate. The configuration shown has a stationary head/moving plate assembly.
- Figure 7: Enclosure for array reactions showing array plate, sliding cover and manifolds for reagent inlet and outlet.

DETAILED DESCRIPTION OF THE INVENTION

The practice of present invention can include a number of photoresist substances. These substances are readily known to those of skill in the art. For example, an optical positive photoresist substance (e.g., AZ 1350 (NovolacTM type-Hoechst CelaneseTM) (NovolacTM is a proprietary novolak resin, which is the reaction product of phenols with formaldehyde in an acid condensation medium)) or an E-beam positive photoresist substance (e.g., EB-9 (polymethacrylate by HoyaTM)) can be used.

A number of siloxane functionalizing reagents can be used, for example:

1. Hydroxyalkyl siloxanes
 - (Silylate surface, functionalize with diborane, and H_2O_2 to oxidize the alcohol)
 - a. allyl trichlorochlorosilane -> -> 3-hydroxypropyl
 - b. 7-oct-1-enyl trichlorochlorosilane -> -> 8-hydroxyoctyl
2. Diol (dihydroxyalkyl) siloxanes
 - (silylate surface, and hydrolyze to diol)
 - a. glycidyl trimethoxysilane -> -> (2,3-dihydroxypropyloxy)propyl

3. Aminoalkyl siloxanes (amines require no intermediate functionalizing step)
 - a. 3-aminopropyl trimethoxysilane -> 3-aminopropyl
4. Dimeric secondary aminoalkyl siloxanes
 - a. bis (3-trimethoxysilylpropyl) amine -> bis (silyloxypropyl) amine

5 In addition, a number of alternative functionalized surfaces can be used in the present invention. These include the following:

1. Polyethylene/polypropylene functionalized by gamma irradiation or chromic acid oxidation, and reduction to hydroxyalkyl surface.
- 10 2. Highly crosslinked polystyrene-divinylbenzene derivatized by chloromethylation, and aminated to benzylamine functional surface.
3. Nylon - the terminal aminohexyl groups are directly reactive.
4. Etched, reduced polytetrafluoroethylene.

There are two important characteristics of the masked surfaces in patterned oligonucleotide synthesis. First, the masked surface must be inert to the conditions
15 of ordinary oligonucleotide synthesis; the solid surface must present no free hydroxy, amino or carboxyl groups to the bulk solvent interface. Second, the surface must be poorly wet by common organic solvents such as acetonitrile and the glycol ethers, relative to the more polar functionalized binding sites.

The wetting phenomenon is a measure of the surface tension or attractive
20 forces between molecules at a solid-liquid interface, and is defined in dynes/cm². Fluorocarbons have very low surface tension because of the unique polarity (electronegativity) of the carbon-fluorine bond. In tightly structured Langmuir-Blodgett type films, surface tension of a layer is primarily determined by the percent of fluorine in the terminus of the alkyl chains. For tightly ordered films,
25 a single terminal trifluoromethyl group will render a surface nearly as lipophobic as a perfluoroalkyl layer. When fluorocarbons are covalently attached to an underlying derivatized solid (highly crosslinked polymeric) support, the density of reactive sites will generally be lower than Langmuir-Blodgett and group density. However, the use of perfluoroalkyl masking agents preserves a relatively high
30 fluorine content in the solvent accessible region of the supporting surface.

There are also two important characteristics of the derivatized regions in patterned oligonucleotide synthesis. The surface must be compatible with the method of detection of hybridization. Radioactivity is largely being replaced by spectroscopic, chemiluminescent and fluorescent detection techniques in DNA
35 research. It is desirable that the surface be optically transparent. A second important characteristic is that the linkage of the penultimate oligonucleotide to the

surface have high chemical stability, at least equal to that of the polyphosphate backbone in DNA.

5 The optical properties of glass (polytetrasiloxane) are unsurpassed for detection purposes. Further, there are numerous techniques developed by the semiconductor industry using thick films (1-5 microns) of photoresists to generate masked patterns of exposed glass surfaces. The best method to derivatize the first exposed glass surface is with volatile fluoroalkyl silanes using gas phase diffusion to create closely packed lipophobic monolayers. The polymerized photoresist provides an effectively impermeable barrier to the gaseous fluoroalkyl silane during 10 the time period of derivatization of the exposed region. Following lipophobic derivatization however, the remaining photoresist can be readily removed by dissolution in warm organic solvents (methyl, isobutyl, ketone, or N-methyl pyrrolidone) to expose a second surface of raw glass, while leaving the first applied silane layer intact. This second region glass can then be derivatized by either 15 solution or gas phase methods with a second, polar silane which contains either a hydroxyl or amino group suitable for anchoring solid phase oligonucleotide synthesis.

Siloxanes have somewhat limited stability under strongly alkaline conditions. Conditions such as 0.1 N sodium hydroxide, typically employed to strip probes from 20 nylon hybridization membranes, should be avoided for reusable glass based hybridization arrays.

Teflon (polytetrafluoroethylene) itself would provide an ideal lipophobic surface. Patterned derivatization of this type of material can be accomplished by reactive ion or plasma etching through a physical mask or using an electron beam, 25 followed by reduction to surface hydroxymethyl groups. However, the opacity of teflon at visible wavelengths severely restrict the applicable methods for detection of hybridization.

Depending on the ultimate application, other organic polymers have desirable characteristics for patterned oligonucleotide synthesis. Polypropylene is relatively 30 transparent to visible light. It can be surface derivatized by chromic acid oxidation, and converted to hydroxy- or aminomethylated surfaces which provide oligonucleotide synthesis anchors of high chemical stability. Highly crosslinked polystyrene-divinylbenzene (ca. 50%) is non-swellable, and can be readily surface derivatized by chloromethylation and subsequent functional group manipulation. 35 Nylon provides an initial surface of hexylamino groups.

The lipophobic patterning of these surfaces can be effected using the same type of solution based thin film masking techniques and gas phase derivatization as glass, or by direct photochemical patterning using o-nitrobenzylcarbonyl blocking groups. Perfluoroalkyl carboxylic and sulfonic acid derivatives rather than silanes are now used to provide the lipophobic mask of the underlying surface during oligonucleotide synthesis.

The solution of chemical reactant can be added to the functionalized binding site through utilization of a piezoelectric pump (Figure 5) in an amount where the solution of chemical reactant at each binding site is separate from the solution of chemical reactant at other binding sites by surface tension. As described more fully *infra*, in the pump depicted in Figure 5, reactant solution is inserted through the inlet (2) into the chamber (6) formed between the upper (1) and lower (5) plates of the piezo. Application of a voltage difference across the upper and lower plates causes compression of the piezo, forcing a microdroplet (4) out through the nozzle (3).

Figure 3 depicts the deposition of the reactant solution on a functionalized binding site and subsequent reaction with the surface. A micro-droplet of solution (Figure 3(a)) is deposited on the functionalized binding site (center cross-hatched region in Figure 3(b)). Because of the differences in wetting properties of the reactant solution on the functionalized binding site and the surrounding surface, the micro-droplet of the reactant solution beads on the functionalized binding site and the reactants in solution react with the surface (Figure 3(c)).

The piezoelectric pump that may be utilized in the invention delivers minute droplets of liquid to a surface in a very precise manner. The pump design is similar to the pumps used in ink jet printing. The picopump is capable of producing 50 micron or 65 picoliter droplets at up to 3000 Hz and can accurately hit a 250 micron target in a 900° C oven at a distance of 2 cm in a draft free environment. Preferred embodiments of the apparatus according to the invention are set forth in Example 3.

Alternative pump designs should take into account the following physical and mechanical considerations for reliable performance to be obtained. When a non-compressible fluid inside of a pumping cavity is subjected to a rapid strong pressure pulse, the direction of flow of the liquid from the cavity is determined primarily by the inertial resistance of the liquid displaced. There is more liquid, and thus resistance to flow, on the inlet side than through the nozzle port. The column of liquid that is forced out of the nozzle begins to neck off as a result of surface

tension. The stream breaks as the piezoelectric is de-energized, with the remaining column of liquid drawn back into the nozzle. The droplet that has necked off continues its flight with the velocity it achieved in the initial acceleration. Typically, the ejection velocity is about 1-2 meters/sec.

5 In normal printing applications using 150 micron drops of viscous water-based inks, the head speed is typically about 0.5 meter/sec. This motion adds a transverse velocity component to the droplet trajectory and can affect aiming accuracy. It may also cause the drop to skip when it hits a surface. Droplets fired from a stationary head tend to evaporate more slowly because they follow in the
10 vapor trail of the preceding drop. The heads work most reliably when the inlet supply lines are not required to flex and the liquids are not subjected to acceleration forces.

The size of the drop is determined primarily by the surface tension of the solution and by the diameter of the pump nozzle. The smaller the droplet, the faster
15 it will evaporate and the more its trajectory will be affected by drafts. Nozzles smaller than 25 microns tend to become plugged with dust particles. For water, the drop diameter is approximately 1.5 times the nozzle diameter. Typically, drops will not vary in size by more than 5%. We have shown that the jet will also successfully eject a variety of polar solvents, including CH_3CN and MeOH . With these less
20 viscous solvents, too forceful an ejection pulse may result in the formation of a series of trailing satellite droplets in addition to the primary drop. The duration of the pulse also affect satelliting.

After the cavity has returned to its original state, a period of time must be allowed for the nozzle to refill by capillary action before another cycle of pulsing
25 can be initiated. It is important for the nozzle refill only to the top of the orifice, but the liquid meniscus not spread out onto the front face of the jet. This is prevented by silanizing the face to reduce its surface tension. The head is also operated under slight negative pressure to prevent overfilling. The aim of the drop is in the axial direction of the nozzle, but defects in the face coating can affect the
30 trajectory.

Arrays of nozzles with up to 64 independent pumping chambers but a common inlet supply have been fabricated. It is important that each chamber inlet have some restriction so that operation of one pumping chamber does not affect the others. The separation between nozzles is typically 400 microns for printing
35 applications, but denser arrays can be produced either by interleaving the transverse motion of the target or decreasing the nozzle spacing.

Example 1

Preparation of Array Plates Ready for Oligonucleotide or Peptide Assembly

The hybridization array is synthesized on a glass plate. The plate is first
5 coated with the stable fluorosiloxane 3-(1,1-dihydroperfluorooctyloxy) propyltrieth-
oxysilane. A CO₂ laser is used to ablate off regions of the fluorosiloxane and expose
the underlying silicon dioxide glass. The plate is then coated with glycidyloxypropyl
trimethoxysilane, which reacts only on the exposed regions of the glass to form a
glycidyl epoxide. The plate is next treated with hexaethyleneglycol and sulfuric
10 acid to convert the glycidyl epoxide into a hydroxyalkyl group, which acts as a
linker arm. The hydroxyalkyl group resembles the 5'-hydroxide of nucleotides and
provides a stable anchor on which to initiate solid phase synthesis. The
hydroxyalkyl linker arm provides an average distance of 3-4 nm between the
oligonucleotide and the glass surface. The siloxane linkage to the glass is completely
15 stable to all acidic and basic deblocking conditions typically used in oligonucleotide
or peptide synthesis. This scheme for preparing array plates is illustrated in Figures
2(A) and 2(B) and was previously discussed.

Example 2

Assembly of Oligonucleotides on the Array Plates

The hydroxyalkylsiloxane surface in the dots has a surface tension of
approximately $\gamma = 47$, whereas the fluorosiloxane has a surface tension of $\gamma = 18$.
For oligonucleotide assembly, the solvents of choice are acetonitrile, which has a
surface tension of $\gamma = 29$, and diethylglycol dimethyl ether. The hydroxyalkyl-
siloxane surface is thus completely wet by acetonitrile, while the fluorosiloxane
25 masked surface between the dots is very poorly wet by acetonitrile. Droplets of
oligonucleotide synthesis reagents in acetonitrile are applied to the dot surfaces and
tend to bead up, as shown in Figure 3. Mixing between adjacent dots is prevented
by the very hydrophobic barrier of the mask. The contact angle for acetonitrile at
the mask-dot interface is approximately $\theta = 43^\circ$. The plate effectively acts as an
30 array microliter dish, wherein the individual wells are defined by surface tension
rather than gravity. The volume of a 40 micron droplet is 33 picoliter. The
maximum volume retained by a 50 micron dot is approximately 100 picoliter, or
about 3 droplets. A 100 micron dot retains approximately 400 picoliter, or about 12
droplets. At maximum loading, 50 micron and 100 micron dots bind about 0.07 and
35 0.27 femtomoles oligonucleotide, respectively.

Assembly of oligonucleotides on the prepared dots (Figure 2B, bottom) is carried out according to the H-phosphonate procedure (Figure 4), or by the phosphoroamidite method. Both methods are well known to those of ordinary skill in the art. Oligonucleotide and Analogs, A Practical Approach (F. Eckstein ed., 1991). Delivery of the appropriate blocked nucleotides and activating agents in acetonitrile is directed to individual dots using the picopump apparatus described in Example 3. All other steps, (e.g., DMT deblocking, washing) are performed on the array in a batch process by flooding the surface with the appropriate reagents. An eight nozzle piezoelectric pump head is used to deliver the blocked nucleotides and activating reagents to the individual dots, and delivering droplets at 1000 Hz, requires only 32 seconds to lay down a 512 x 512 (262k) array. Since none of the coupling steps have critical time requirements, the difference in reaction time between the first and last droplet applied is insignificant.

Example 3

Construction of Piezoelectric Impulse Jet Pump Apparatus

Piezoelectric impulse jets are fabricated from Photoceram (Corning Glass, Corning, N.Y.), a UV sensitive ceramic, using standard photolithographic techniques to produce the pump details. The ceramic is fired to convert it to a glassy state. The resulting blank is then etched by hydrogen fluoride, which acts faster in exposed than in nonexposed areas. After the cavity and nozzle details are lapped to the appropriate thickness in one plate, the completed chamber is formed by diffusion bonding a second (top) plate to the first plate. The nozzle face is lapped flat and surface treated, then the piezoelectric element is epoxied to the outside of the pumping chamber. When the piezoelectric element is energized it deforms the cavity much like a one-sided bellows, as shown in Figure 5.

To determine the appropriate orifice size for accurate firing of acetonitrile droplets, a jet head with a series of decreasing orifice sizes is prepared and tested. A 40 micron nozzle produces droplets of about 65 picoliter.

A separate nozzle array head is provided for each of the four nucleotides and a fifth head is provided to deliver the activating reagent for coupling. The five heads are stacked together with a mechanically defined spacing. Each head has an array of eight nozzles with a separation of 400 microns.

The completed pump unit is assembled with the heads held stationary and the droplets fired downward at a moving array plate as shown in Figure 6. The completed pump unit assembly (3) consists of nozzle array heads (4-7) for each of the

four nucleotidase and a fifth head (8) for activating reagent. When energized, a microdroplet (9) is ejected from the pump nozzle and deposited on the array plate (1) at a functionalized binding site (2).

5 A plate holding the target array is held in a mechanical stage and is indexed in the X and Y planes beneath the heads by a synchronous screw drives. The mechanical stage is similar to those used in small milling machines, microscopes and microtomes, and provides reproducible positioning accuracy better than 2.5 microns or 0.1 mil. As shown in Figure 7, the plate holder (3) is fitted with a slotted spacer (4) which permits a cover plate (5) to be slid over the array (6) to form an enclosed
10 chamber. Peripheral inlet (1) and outlet (2) ports are provided to allow the plate to be flooded for washing, application of reagents for a common array reaction, or blowing the plate dry for the next dot array application cycle.

Both the stage and head assembly are enclosed in a glove box which can be evacuated or purged with argon to maintain anhydrous conditions. With the plate
15 holder slid out of the way, the inlet lines to the heads can be pressurized for positive displacement priming of the head chambers or flushing with clean solvent. During operation, the reagent vials are maintained at the ambient pressure of the box.

With a six minute chemistry cycle time, the apparatus can produce 10-mer array plates at the rate of 1 plate or 10^6 oligonucleotides per hour.

20 Example 4

Use of Oligonucleotide Array Plates to Determine the Nucleotide Sequence of a Target Nucleic Acid

The oligonucleotide array plate is prepared as described in Examples 1 and 2, using the apparatus described in Example 3. The array contains oligonucleotides
25 having 10 nucleotides each (10-mers). The synthesis is carried out such that each oligonucleotide element, moving in a 5'-3' direction, is identical to the preceding element in nucleotide sequence, except that it deletes the 5'-most nucleotide, and adds a new 3'-most oligonucleotide. In this way the total array represents every possible permutation of the 10-mer oligonucleotide. Oligonucleotides are spaced at
30 7 nm intervals to provide an oligonucleotide loading density of 3.4×10^{-12} moles/cm², or 2.6×10^{-16} moles per 100 micron element. The target nucleic acid is used to probe the oligonucleotide array plate. The probe is labelled with 1000 Ci/nmol P³². The labelled probe is contacted with the oligonucleotide array plate for hybridization in
35 a 10nM solution of probe in 3M Me₄NCl at 42°C. At 10% hybridization and wash efficiency, each oligonucleotide element dot having an exact match with the probe binds 26 attomoles of probe. Radiolabel binding is detected using a Bio-Image

AnalyzerTM (Fuji, Waltham, MA). The pattern of binding is assessed and the nucleotide sequence of the probe nucleic acid is determined by ordering the nucleotide sequence according to the known sequences of the oligonucleotide elements, as shown in Figure 1.

- 5 Figure 1 depicts a sequencing arrangement based on a matrix of trimer oligonucleotides bound to the array plate. Figure 1(a) is the basic matrix consisting of the four nucleotides. Figure 1(b) is the complete trimer matrix, representing each of the 4^3 trimer permutations. The underlined elements in the array represent sites to which the target nucleic acid is bound. Figure 1(c) depicts how a sequence
10 complementary to the target nucleic acid is constructed from the known sequences of the sites to which the target nucleic acid is bound.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Brennan Ph.D., Thomas M.

5 (ii) TITLE OF INVENTION: Method and Apparatus for Conducting an
Array of Chemical Reactions on a Surface

(iii) NUMBER OF SEQUENCES: 1

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: John J. McDonnell, Allegretti & Witcoff, Ltd.

10 (B) STREET: 10 South Wacker Drive, Suite 3000

(C) CITY: Chicago

(D) STATE: IL

(E) COUNTRY: USA

(F) ZIP: 60606

(v) COMPUTER READABLE FORM:

15 (A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

20 (A) APPLICATION NUMBER: US 08/068,540

(B) FILING DATE: 27-MAY-1993

(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

25 (A) NAME: McDonnell Ph.D., John J.

(B) REGISTRATION NUMBER: 26,949

(C) REFERENCE/DOCKET NUMBER: 91,781-A

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (312)715-1000

(B) TELEFAX: (312)715-1234

30 (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 base pairs

(B) TYPE: nucleic acid

35 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATTCTTGTTA

10

What is claimed is:

1. A method for conducting chemical reactions between a solution of a chemical reactant and an array of functionalized binding sites on a support surface comprising adding the solution of chemical reactant to the functionalized binding site in an amount where the solution of chemical reactant at each binding site is separate from the solution of chemical reactant at other binding sites by surface tension.
2. The method of claim 1 wherein the area of the support surface of the functionalized binding site has a higher surface tension relative to the support surface surrounding each functionalized binding site.
3. The method of claim 1 wherein the support surface has $10\text{-}10^4$ functionalized binding sites per cm^2 .
4. The method of claim 1 wherein the functionalized binding sites are about 50-2000 microns in diameter.
5. The method of claim 1 wherein the volume of the solution of reagent(s) is 50 picoliter to 2 microliter.
6. The method of claim 1 wherein the chemical reaction between the chemical reactant and functionalized binding site forms covalent bonds.
7. The method of claim 1 wherein chemical reactant reacts with the functionalized binding site by non-covalent specific binding interactions.

8. An array plate comprising a support surface with an array of distinct and separated functionalized binding sites and wherein the area of the functionalized binding sites has a higher surface tension relative to the support surface surround each functionalized binding site.

9. The array plates of claim 8 wherein the support surface has 10 to 10^4 sites/cm² functionalized binding sites per cm².

10. The array plate of claim 8 wherein each functionalized binding site is about 50-2000 microns in diameter.

11. The array plate of claim 8 wherein the functionalized binding sites are functionalized with a reagent which forms a covalent-chemical bond with the reagent.

12. The array plate of claim 8 wherein the functionalized binding sites are functionalized with a reagent that is a member of a specific binding pair.

13. A method for making array plates comprising:

(a) coating a support surface with a positive or negative photoresist substance which is subsequently exposed to light and developed to create a patterned region of a first exposed support surface;

(b) reacting the first support surface with a fluoroalkylsilane to form a stable fluoroalkylsiloxane hydrophobic matrix on the first support surface;

- (c) removing the remaining photoresist to expose a second support surface; and
 - (d) reacting the second support with a hydroxy or aminoalkylsilane to form derivatized hydrophilic binding site regions.
14. The method according to claim 13, wherein the fluoroalkylsiloxane is tetradecafluoro-1,1,2,2-tetrahydrooctyl siloxane.
15. A method for making array plates comprising:
- (a) reacting a support surface with a hydroxy or aminoalkylsilane to form a derivatized hydrophilic support surface;
 - (b) reacting the support surface from step (a) with o-nitrobenzyl carbonyl chloride as a temporary photolabile blocking to provide a photoblocked support surface;
 - (c) exposing the photoblocked support surface of step (b) to light through a mask to create unblocked areas on the support surface with unblocked hydroxy or aminoalkylsilane;
 - (d) reacting the exposed surface of step (c) with perfluoroalkanoyl halide or perfluoroalkylsulfonyl halide to form a stable hydrophobic (perfluoroacyl or perfluoroalkylsulfonamido) alkyl siloxane matrix; and
 - (e) exposing this remaining photoblocked support surface to create patterned regions of the unblocked hydroxy- or aminoalkylsilane to form the derivatized hydrophilic binding site regions.
16. The method according to claim 15, wherein the siloxane is 3-perfluorooctanoyloxy propylsiloxane.

17. The method according to claim 15 wherein the siloxane is 3-perfluorooctanesulfonamido propylsiloxane.

FIG.1a { A C
G T Matrix

AAA AAC ACA ACC CAA CAC CCA CCC
AAG AAT ACG ACT CAG CAT CCG CCT
AGA AGC ATA ATC CGA CGC CTA CTC
AGG AGT ATG ATT CGG CGT CTG CTT
GAA GAC GCA GCC TAA TAC TCA TCC
GAG GAT GCG GCT TAG TAT TCG TCT
GGA GGC GTA GTC TGA TGC TTA TTG
GGG GGT GTG GTT TGG TGT TTG TTT

FIG.1b

DNA Fragment

-----ATTCTTGTTA----

ATT
TTC
TCT
CTT
TTG
TGT
GTT
TTA

TTA,TTG

TTA

FIG.1c

Correct Assembly Possible N+1 List

FIG. 2A

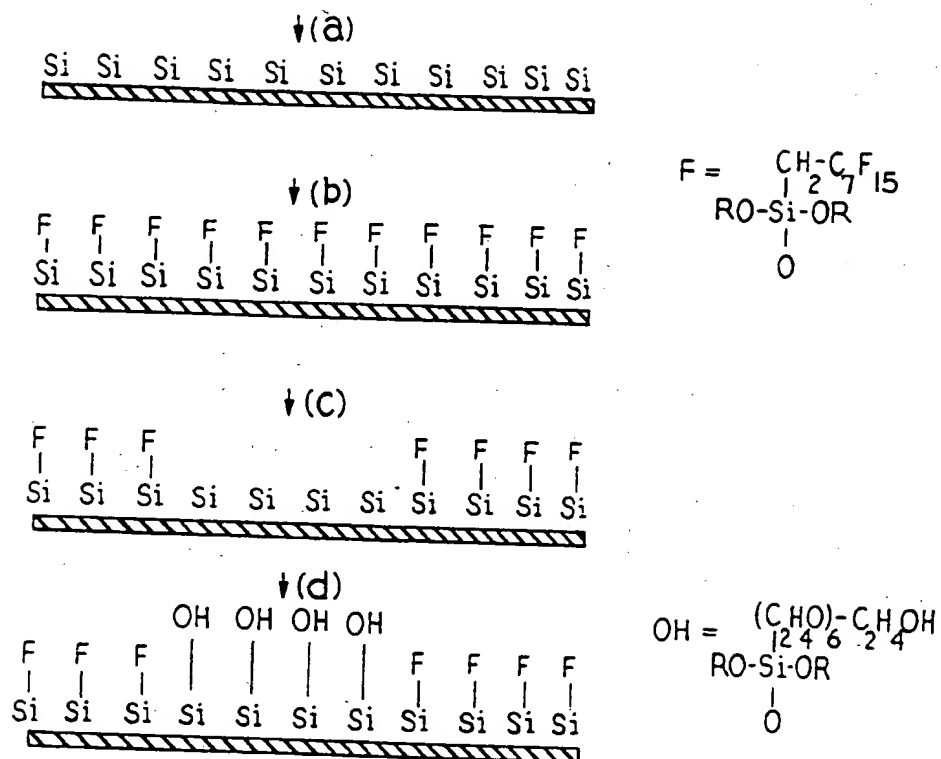


FIG.2B O-Nitrobenzyl Carbamate Array Masking Chemistry

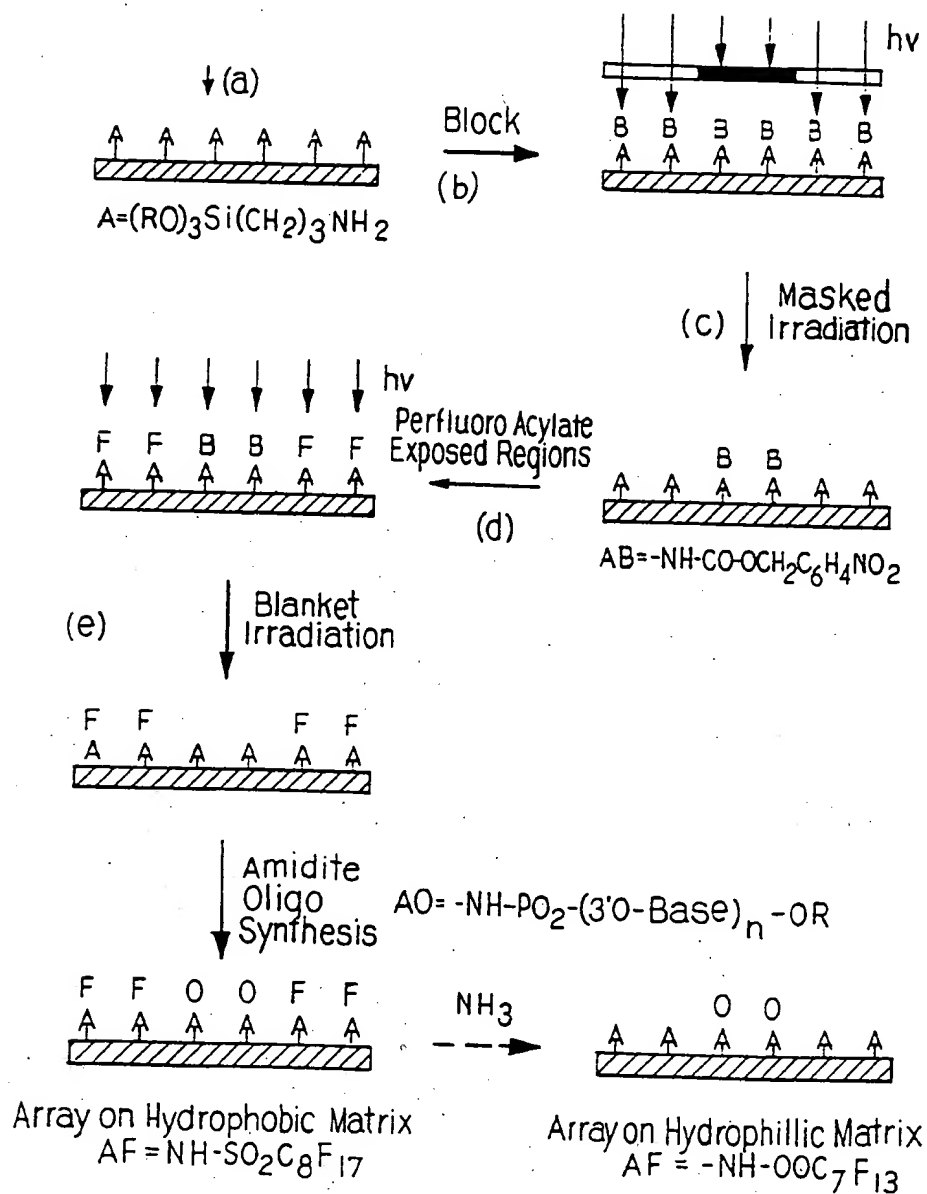


FIG. 3

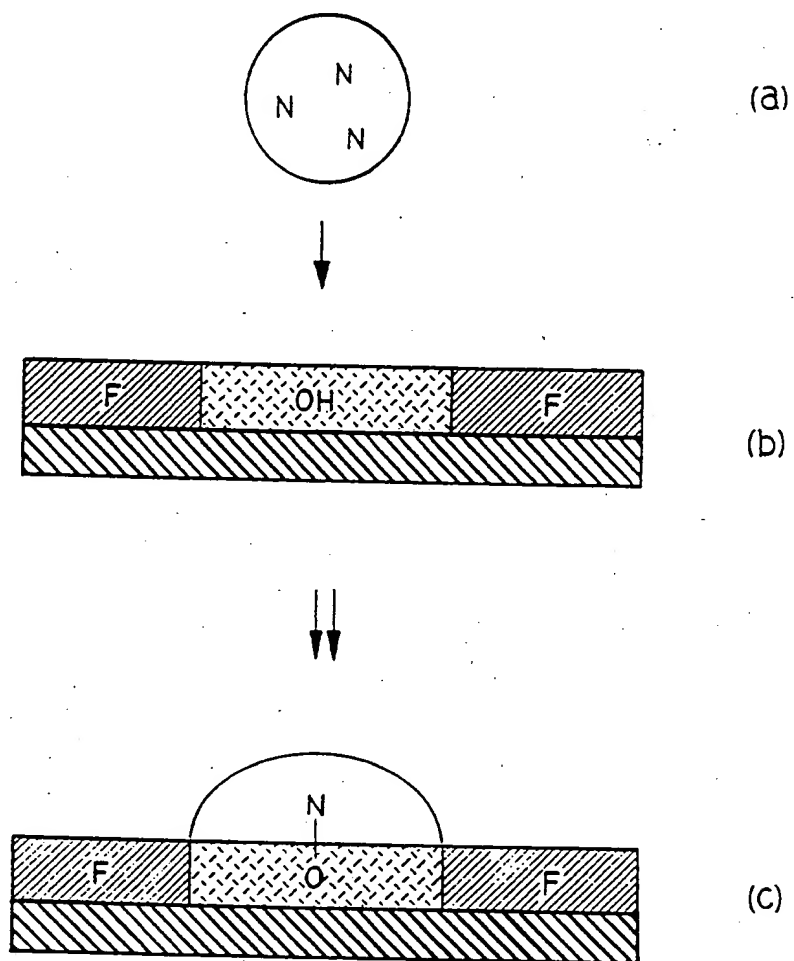


FIG. 4

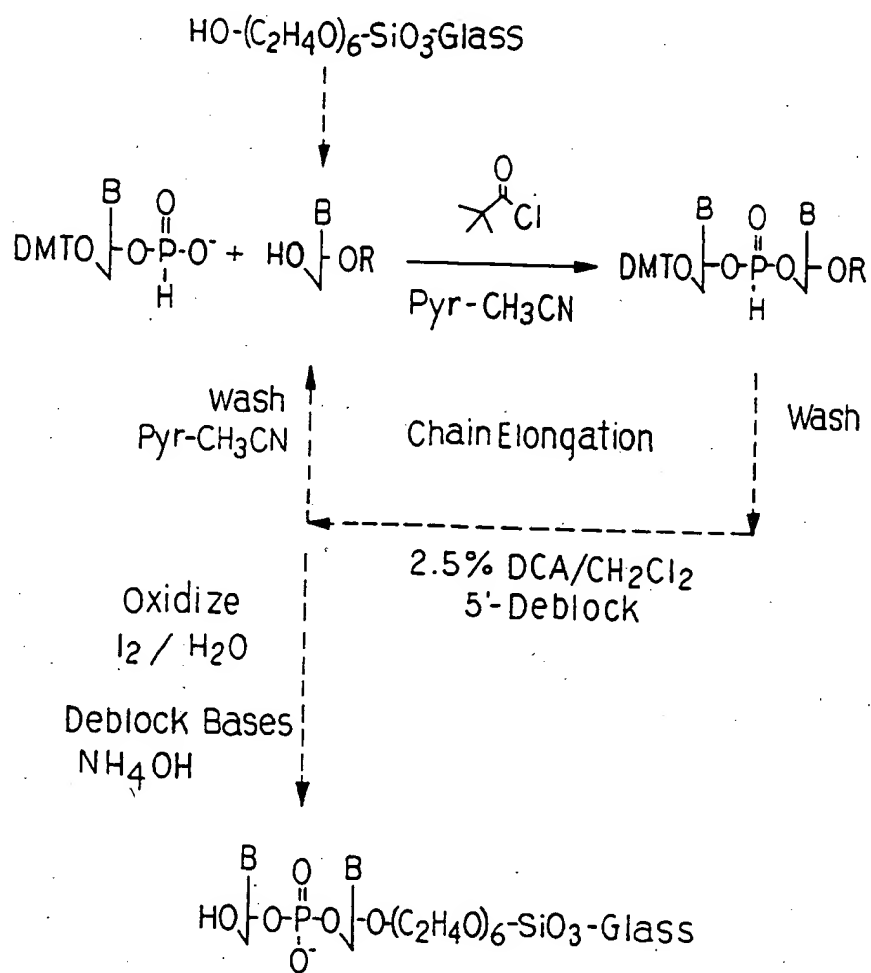


FIG. 5a

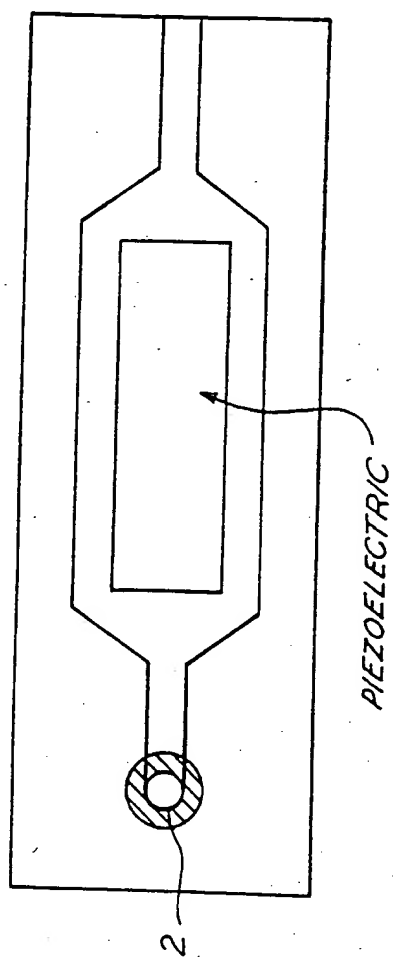
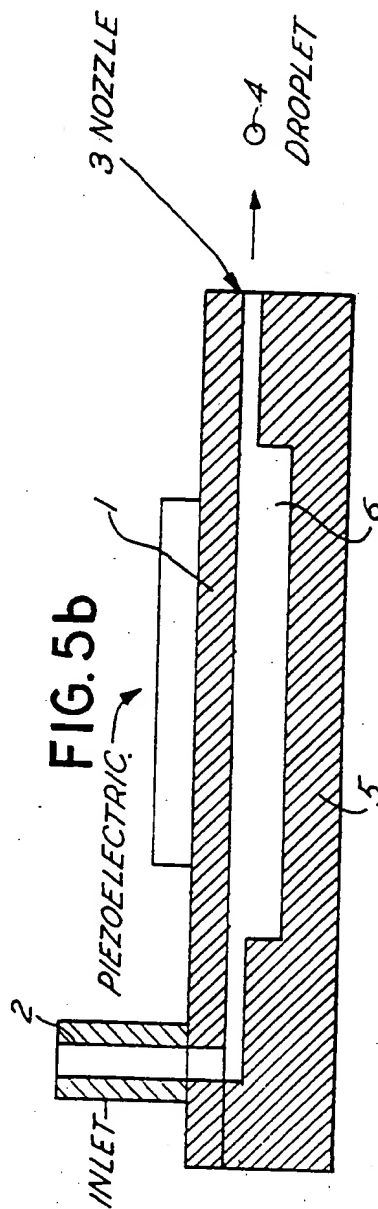


FIG. 5b



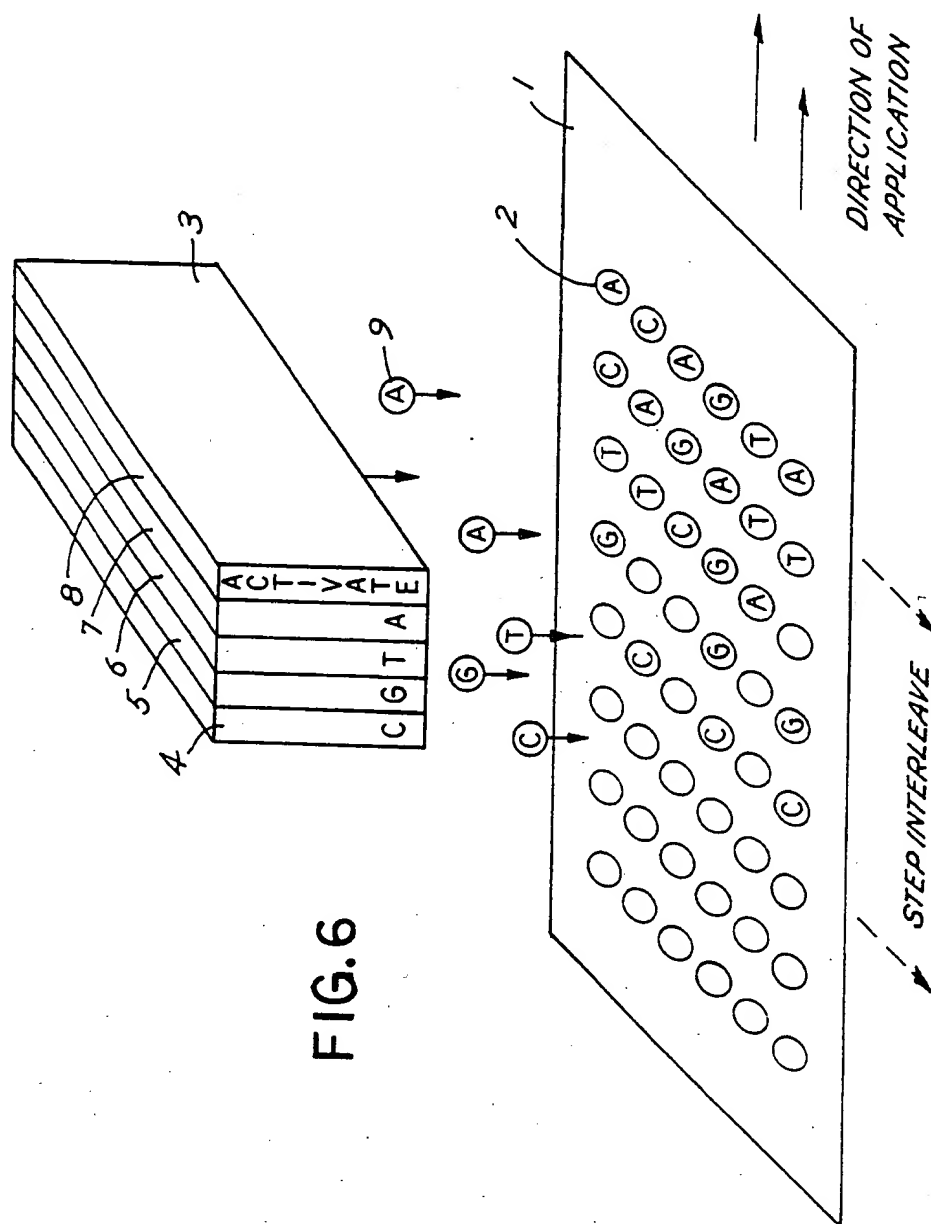
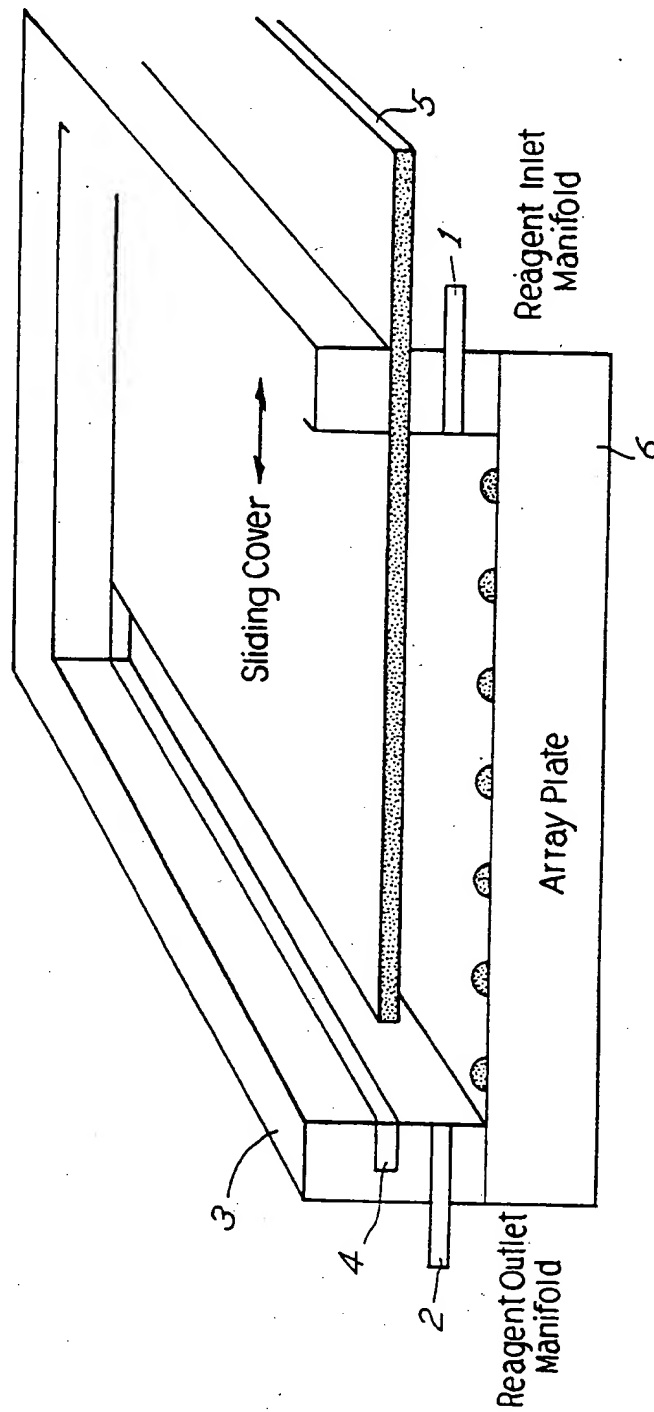


FIG. 7



INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER IPC 5 B01J19/00 C07H21/00 C03C17/30 C07B61/00 C07K1/04		Int. Application No. PCT/US 94/05896
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 5 B01J C03C C07B C07H C07K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 161 058 (DOW CORNING CORPORATION) 13 November 1985 see the whole document ---	1
A	WO,A,90 15070 (AFFYMAX TECHNOLOGIES N.V.) 13 December 1990 see the whole document ---	1
A	WO,A,90 03382 (ISIS INNOVATION LIMITED) 5 April 1990 cited in the application see the whole document ---	1
-/--		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
Date of the actual completion of the international search 29 August 1994		Date of mailing of the international search report 05.09.94
Name and mailing address of the ISA European Patent Office, P.O. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fac (+ 31-70) 340-3016		Authorized officer Rinkel, L

INTERNATIONAL SEARCH REPORT

Int. Application No.
PCT/US 94/05896

(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>SCIENCE, vol. 251, 1991 pages 767 - 773 FODOR, S.P.A. ET AL. 'LIGHT-DIRECTED, SPATIALLY ADDRESSABLE PARALLEL CHEMICAL SYNTHESIS' cited in the application see the whole document -----</p>	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 94/05896

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0161058	13-11-85	CA-A- 1245208	22-11-88
		JP-A- 60231741	18-11-85
		US-A- 5110784	05-05-92
WO-A-9015070	13-12-90	US-A- 5143854	01-09-92
		AU-B- 651795	04-08-94
		AU-A- 5837190	07-01-91
		CA-A- 2054706	08-12-90
		EP-A- 0476014	25-03-92
		GB-A, B 2248840	22-04-92
		JP-T- 4505763	08-10-92
		NL-T- 9022056	02-03-92
WO-A-9003382	05-04-90	DE-D- 68914138	28-04-94
		DE-T- 68914138	30-06-94
		EP-A- 0386229	12-09-90
		JP-T- 4500671	06-02-92